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Growth characteristics of *Escherichia coli* O157:H7 on sliced meat at temperature abuse conditions

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This study investigated the growth and survival of *Escherichia coli* O157:H7 and aerobic bacteria (AB) in air (AP), vacuum (VP) and modified atmosphere packed (MAP) with sliced meat stored at 4, 6, 8 and 12°C. A slight reduction in the numbers of *E. coli* O157:H7 was observed after 21 days at 4°C in the VP and MAP sliced meat while significant ($p<0.05$) growth of bacterium was observed with storage temperature increased from 6 to 12°C in all packed sliced meats. Growth of AB was observed during storage from 4 to 12°C in all packed sliced meats. Lag phase duration of *E. coli* O157:H7 and AB at 4-12°C were significantly ($p<0.05$) higher in the AP sliced meat than VP and MAP. The growth rates of *E. coli* O157:H7 and AB on the sliced meat stored at 6-12°C were significantly ($p<0.05$) higher than those of *E. coli* O157:H7 stored at 4°C. Temperature control is very important to ensure no growth of *E. coli* O157:H7 on the sliced meat with modification of storage atmosphere.

Key words: *Escherichia coli* O157:H7, aerobic bacteria, meat, vacuum packaging, modified atmosphere packaging, temperature.

INTRODUCTION

The increase in world population and change in lifestyles have resulted in demands on animal origin foods. Foods may be contaminated by different pathogenic and spoilage microorganisms causing food spoilage and human health problems (Erkmen and Bozoglu, 2008b). *Escherichia coli* makes up a large proportion of the intestinal microflora of gastrointestinal tract of animals. The vehicle of *E. coli* O157:H7 infection was thought to be meat and meat products from a butcher's shop (Dykes et al., 2001). Surface cross-contamination of foodborne pathogens in food products during processing or preparation is a major concern to consumers and food manufacturers. Although risk assessment and analyses have been applied to monitor and reduce the hazards (Graumann and Holley, 2007; Erkmen and Bozoglu, 2008b). Pathogenic *E. coli* strains have emerged as an important pathogen, causing worldwide disease and economic loss. The infection caused by *E. coli* O157:H7 has been reported from many countries (Mead and Griffin, 1998; Arias et al., 2001; Blanco et al., 2003).

Surface foodborne pathogen transfer during slicing is one of the important factors to impact the food safety in preparing sliced meat. A slicer is commonly used and most likely to be the last preparation step before packaging or wrapping of the meat products. These products are available in the supermarket refrigeration section and produced either by brand-named manufacturers or in store preparation, including made-to-order. Sliced meats are also commonly sold in delicatessen and fast food restaurants, where a retail-scale slicer is often used on site for meal or sandwich preparations. The slicing equipment, if not properly cleaned and sanitized, can cause microbial cross-contamination (Dubal et al., 2004; Perez-Rodriguez et al., 2007; Erkmen and Bozoglu, 2008b).

Chilled storage of fresh beef under vacuum or carbon dioxide (CO_2) atmospheres is used commercially to extend

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product storage (Erkmen and Bozoglu, 2008a). Pathogen behavior on raw meats, as with many other foods, is controlled by several interrelated factors, such as temperature of storage, atmosphere composition and competitive microflora (ICMSF, 1998; Erkmen and Barazi, 2008; Erkmen and Bozoglu, 2008a). The current assumption is that *E. coli* O157:H7 does not multiply below 7°C, which is the endpoint generally accepted by the European Union as an appropriate temperature to prevent mesophilic pathogen growth (Anon, 2004). However, conditions at both retail and wholesale level can provide an opportunity for numbers to increase because of temperature abuse or temperature fluctuations (ICMSF, 2002; Zhou et al., 2010). *E. coli* O157:H7 is a potential contaminant in the environment especially when the slicing equipment/ operation involve multiple products (Graumann and Holley, 2007). In this study, the growth characteristics of *E. coli* O157:H7 and aerobic bacteria (AB) in air, vacuum and modified atmosphere packed sliced meat stored at different temperatures (4, 6, 8 and 12°C) were investigated. The growth parameters were also indicated to describe the survival of *E. coli* O157:H7 and AB during storage of packed sliced meat.

MATERIALS AND METHODS

Escherichia coli O157:H7 preparation

A cocktail of two strains was used for the surface contamination of *E. coli* O157:H7 on sliced meat. *E. coli* O157:H7 NCTC 12900 and NCTC 13126 were obtained from The National Collection of Type Cultures (Porton Down, Salisbury, SP4 0JG, UK). Each strain was transferred from a stock culture kept at -27°C into 10 ml of brain heart infusion broth (BHIB; Difco, Detroit) and incubated at 35°C for 18 h. Zero-point 1 ml of each culture was also transferred into another ten ml BHIB and again incubated at 35°C for 18 h. Ten ml of each *E. coli* O157:H7 culture was mixed together in a 5 L bottle containing sterile 0.1% peptone water (PW). That was used as *E. coli* O157:H7 contamination culture. The number of *E. coli* O157:H7 in contamination culture was counted by spread plating 1 ml of sample in duplicate onto the surface of sorbitol-MacConkey agar plates supplemented with cefixime (0.5 mg l^{-1}) and potassium tellurite (2.5 mg l^{-1}) (CT-SMAC, Oxoid, USA). The number of *E. coli* O157:H7 in contamination culture was about $3.84x10^4$ colony forming units (cfu)/ml. Contamination culture was used for contaminating *E. coli* O157:H7 to surface area of sliced meat. A separate contamination culture was prepared for each replicate.

Meat preparation, packaging and storage conditions

Fresh sliced raw beef meat (average cross-cut surface approximately 6x3 cm with approximately 0.5 cm thick) with 14% fat was obtained from a local butcher and transported to the laboratory in cooler boxes containing ice packs. The initial pH of sliced meat was about 5.72.

Samples of sliced meat from each replicate were screened for the presence of naturally occurring *E. coli* O157:H7 using the following procedures. Briefly, 25 g of sliced meat was added into 500 ml flask containing 225 ml sterile EC (*E. coli*) broth (Oxoid, USA) supplemented with 0.02 mg ml^{-1} novobiocin (Sigma-Aldrich, USA) and incubated for 18-24 h at 37°C. Then, the culture was streaked out onto CT-SMAC plates and incubated for 18-24 h at 37°C.

Sorbitol-negative colonies were streaked for purity and screened by the indole test and other confirmation tests for *E. coli* O157:H7 (Erkmen, 2007).

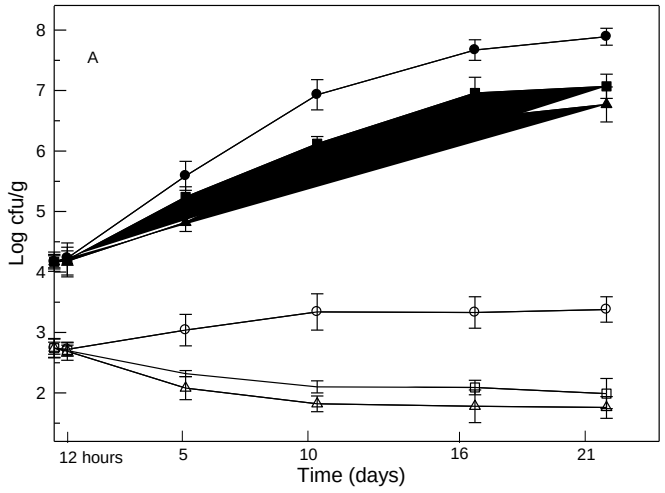
About five kg sliced meat pieces were contaminated by immersion in the 10 L bottle containing contamination culture for 2 min. The contents of bottle were filtered through sterile cheesecloth under aseptic conditions and then meat pieces were placed into other sterile cheesecloth and allowed to drain for 5 min. The *E. coli* O157:H7 contamination level on sliced meat was about $5.25x10^2$ cfu/g. About 40 g of *E. coli* O157:H7 contaminated sliced meat was placed into sterile polyethylene/polyamide film (PE/PE) (Polinas Plastic Industry and Trade Inc., Manisa, Turkey) packages (20x12 cm) under aseptic condition. According to the manufacturer's data, PE/PA film has permeability at 25°C; oxygen: 160 $cm^3m^{-2}day^{-1}$ and water vapor: 8.5 g $m^{-2}day^{-1}$. The *E. coli* O157:H7 contaminated sliced meat was (i) air packed (AP), (ii) vacuum packed (VP) and (iii) modified atmosphere packed (MAP) (10% O_2+40% CO_2+50% N_2) in vacuum packing machine (La Minerra, D.V.P. Vacuum Tech-nology, s.r.l., Italy) within 5 min at 20°C. The CO_2, O_2 and N_2 were mixed using Witt-Gas mixer (GmbH and Co Kg, Deutschland) and the gas mixture was flushed into vacuum machine during packaging. Control packages were also prepared from non-contaminated sliced meat (without *E. coli* O157:H7 contamination). At least 10 packages from each packaging type were stored at each 4, 6 and 8°C for 21 days, and at 12°C for 16 days with a relative humidity of 85-90%.

Sampling and microbiological analysis

Two packages from each packaging type were taken on sampling days during storage. The sampling frequency was every 12 h at the beginning of storage and on 5, 10, 16 and 21 days of storage after growth of *E. coli* O157:H7 was observed. *E. coli* O157:H7 and AB were counted from sliced meat samples using the following procedures. Briefly, 25 g of sliced meat from package was added into 500 ml flask containing 225 ml sterile 0.1% PW, the flask was placed to a shaker (ST-402, Nüve, Industry and Equipment Manufacturing and Trade Inc., İstanbul) and shaked for 5 min. Sample dilutions were spread-plated in duplicate on CT-SMAC and brain heart infusion agar (BHIA; Difco, Detroit), and plates were incubated at 35°C for 24 h to count *E. coli* O157:H7 and AB respectively. The AB counts from control meat samples (without *E. coli* O157:H7 inoculation) and before inoculation *E. coli* O157:H7 onto the sliced meats were also determined. Characteristic colorless (or neutral/ gray) smoky center in 1-2 mm diameter colonies from plates containing 30 to 300 colonies were counted as *E. coli* O157:H7 (Erkmen, 2007). Least three characteristic *E. coli* O157:H7 colonies per plate are confirmed by morphological and biochemical tests (Erkmen, 2007). All bacterial colonies from BHIA plates were counted from plates. The number of survivors was expressed as log cfu/g. Each experimental condition was repeated three times. Each value represents the mean of six values (duplicate values for each sample analyzed from three independent trials).

Growth parameters of *E. coli* O157:H7 and AB on the sliced meat

The model selected is a three-phase line one that divides the growth curve into the lag, exponential and stationary growth phases by using SigmaPlot v.10 for windows (Statsoft, Chicago IL, USA). During the lag phase, the cells are assumed to be replicating, as they adapt themselves to their new environment. Once adapted, the cells begin to grow at a rate that is maximal for the microorganism in the specific environment. During the exponential growth phase, the specific growth rate assumed to be a constant (μ=k) with the log time. Once the stationary phase has been reached, there is no net increase in number and the specific growth rate returns to zero (μ =



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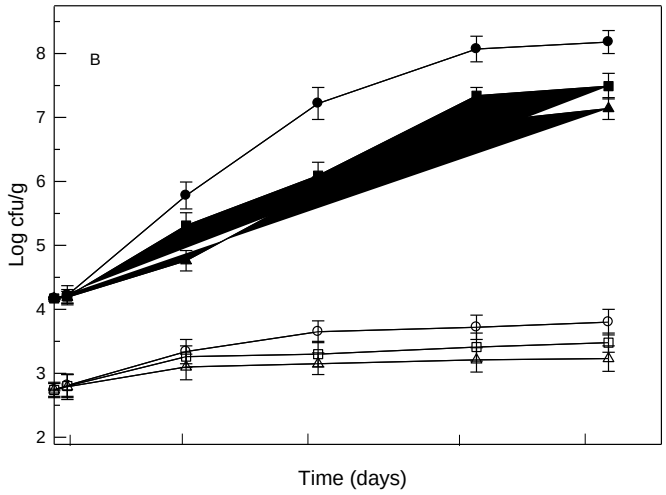

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Figure 1. Growth of *E. coli* O157:H7 and aerobic bacteria (AB) during storage in the packed sliced meat at 4°C (A) and 6°C (B). (o) AP-*E. coli*, (•) AP-AB, (□) VP-*E. coli*, (■) VP-AB, (Δ) MAP-*E. coli* and (▲) MAP-AB.

0) (Buchanan et al., 1997; Erkmen and Bozoglu, 2008a; Hwang and Sheen, 2011). The numbers (log cfu/g) of *E. coli* O157:H7 and AB on the sliced meat during storage at 4, 6, 8 and 12°C were used to obtain the lag phase duration (LPD, h), growth rate (GR, log cfu/h) and maximum microbial number (MMN, log cfu/g):

Lag phase: For $t \leq t_{lag}$, $N_t = N_0$,
Exponential growth phase: For $t_{lag} < t < t_{max}$, $GR = (N_t - N_0)/(t - t_{lag})$,
Stationary phase: For $t \geq t_{max}$, $N_t = N_{max}$,

Where N_0 is the initial numbers (log cfu/g), N_t is the bacterial numbers at time t (h), N_{max} is the maximum bacterial numbers, t_{lag} is the lag phase duration (h), t_{max} is the time required for bacterial numbers to reach the maximum (N_{max}).

Statistical analysis

Microbiological data were statistically analyzed (mean, standard deviation, variance and correlation coefficient) using the SigmaPlot v.10. Analysis of variance (ANOVA) of the data was carried out on the mean logarithmic to the base 10 cfu/g at each temperature-time for packed sliced meat at the 95 % confidence level. Analysis of variance was applied to the parameters (GRs, LPDs and MMNs of *E. coli* O157:H7 and AB) to determine statistical differences using the t-test among and between different storage conditions (temperatures, packaging types and storage period).

RESULTS

Growth of *E. coli* O157:H7 and AB on the sliced meat

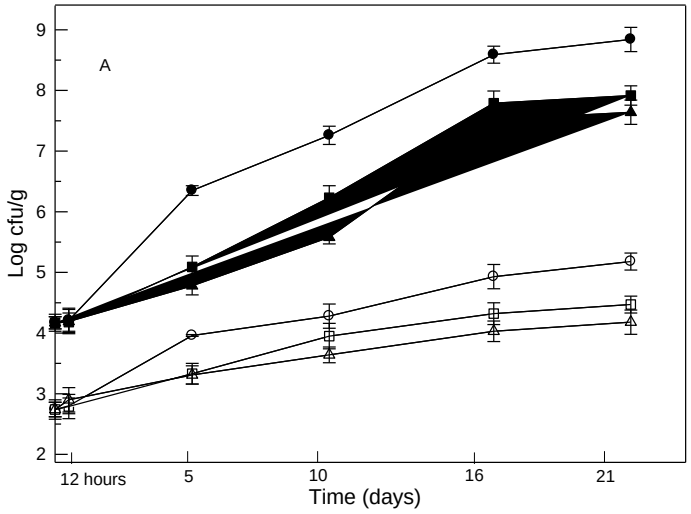
E. coli O157:H7 was not detected on any un-inoculated sliced meat. The pH of sliced meat samples at the end of storage were 0.3-0.5 higher than the initial pH. The initial numbers of *E. coli* O157:H7 and AB on the sliced meat were 2.74 and 4.17 log cfu/g respectively. The changes in the number of microorganisms were very low at 4, 6, 8 and 12°C after 12 h storage in the AP, VP and MAP sliced meat (Figures 1 and 2).

At 4°C, no growth of *E. coli* O157:H7 was observed in the VP and MAP sliced meat (Figure 1A). It was slightly (0.64 cfu/g) increased after 21 days of storage in the AP sliced meat. Slight reduction in the number of *E. coli* O157:H7 was observed during storage, as the initial level of the bacterium decreased to 1.99 and 1.76 log cfu/g in the VP and MAP sliced meat respectively after 21 days. There were no significant ($p>0.05$) differences in the number of *E. coli* O157:H7 in the VP and MAP sliced meat during storage.

At 6°C, the growth of *E. coli* O157:H7 was observed in the AP, VP and MAP sliced meat during 21 days storage (Figure 1B), as the *E. coli* O157:H7 counts increased to 3, 80, 3.48 and 3.23 log cfu/g respectively. During 3 days storage, the levels of *E. coli* O157:H7 remained constant in the AP sliced meat. During the next 7 days, growth was slightly increased and then remained constant. There were no significant ($p>0.05$) differences in the number of *E. coli* O157:H7 during 21 days storage in the AP, VP and MAP sliced meat.

At 8°C, constant growth of *E. coli* O157:H7 was observed in the AP, VP and MAP sliced meat during 21 days storage (Figure 2A), as the *E. coli* O157:H7 counts increased to 5.18, 4.47 and 4.18 log cfu/g respectively. The levels of *E. coli* O157:H7 remained constant during 3 days, increased during next 7 days and slower during remaining storage period. Significant ($p<0.05$) growth of *E. coli* O157:H7 was observed in the AP, VP and MAP sliced meat during 21 days storage. In the AP sliced meat, *E. coli* O157:H7 counts were significantly ($p<0.05$) higher than those observed in the VP and MAP sliced meat. This indicated that the absence of air in the package reduced the growth of the pathogen.

At 12°C, growth of *E. coli* O157:H7 was observed in the AP, VP and MAP sliced meat during 16 days storage



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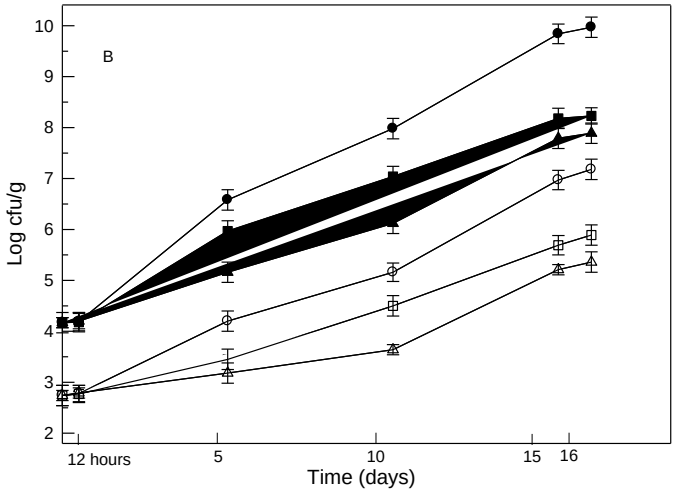

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Figure 2. Growth of *E. coli* O157:H7 and aerobic bacteria (AB) during storage in the packed sliced meat at 8°C (A) and 12°C (B). (o) AP-*E. coli*, (•) AP-AB, (□) VP-*E. coli*, (■) VP-AB, (Δ) MAP-*E. coli* and (▲) MAP-AB.

(Figure 2B). Significant ($p<0.05$) growth of *E. coli* O157 : H7 was observed in all packed sliced meat. The growth of *E. coli* O157:H7 was higher in the AP sliced meat than the VP and MAP. By the day 16, *E. coli* O157:H7 numbers reached their highest number in the AP, VP and MAP sliced meat, as increased to 7.18, 5.89 and 5.36 log cfu/g respectively.

At 4°C (Figure 1A), the number of AB was remained constant during 3 days of storage in the AP, VP and MAP sliced meat while slight growth was observed at 6°C (Figure 1B). There was rapid increase during next 13 days and the growth was slower in last 5 days. AB counts increased when the temperature increased from 4 to 6°C. The highest number of AB was observed ($p<0.05$) in AP sliced meat than VP and MAP, as increased to 8.18, 7.49 and 7.14 log cfu/g, respectively, at 6°C after 21 days. This indicated that the absence of air in the package can reduced the growth of AB. Significant ($p<0.05$) growth of AB was observed in the AP, VP and MAP sliced meat during 21 days storage at 6°C. Significant ($p<0.05$) differences on the growth of AB were between 4 and 6°C.

Under 8 (Figure 2A) and 12°C (Figure 2B) storage, the growth of AB continuously increased during storage period in all packed sliced meat. However, the growth remained constant in the last 5 days at 8°C and 2 days at 12°C. The numbers of AB increased to 9.97, 8.23 and 7.89 log cfu/g at 12°C after 16 days in the AP, VP and MAP sliced meat, respectively, while the numbers increased to 8.84, 7.91 and 7.64 log cfu/g at 8°C in the AP, VP and MAP sliced meat, respectively. The increase in the AP sliced meat was significantly ($p<0.05$) higher than the VP and MAP sliced meat. Growth of AB at 12°C was significantly ($p<0.05$) higher than at 8°C in all packages.

Growth characteristics of *E. coli* O157:H7 and AB on the sliced meat

The LPDs of *E. coli* were 75, 69, 50 and 37 h in the AP sliced meat stored at 4, 6, 8 and 12°C, respectively (Table 1). In the VP sliced meat, the LPDs of *E. coli* O157:H7 were 81, 72, 55 and 42 h at respective temperatures. The LPDs of *E. coli* O157:H7 were not significantly ($p>0.05$) different at all storage temperatures of the AP, VP and MAP sliced meat. The GRs for *E. coli* O157:H7 in the VP and MAP sliced meat stored from 4 to 12°C were ranged from -0.0015 to 0.0063 and -0.0019 to 0.0052 log cfu/h, respectively (Table 1) while packed with air was ranged from 0.0013 to 0.0088 log cfu/h. The GRs of *E. coli* O157:H7 or AB in the packed sliced meat stored at 6, 8 and 12°C were significantly higher ($p<0.05$) than at 4°C. The GRs of *E. coli* O157:H7 and AB in the each type of packed sliced meat were significantly ($p<0.05$) increased with the temperature increase from 4 to 12°C. During storage at 12°C, the MMNs of *E. coli* O157:H7 and AB is reached to 7.18 and 9.97 log cfu/g, respectively, in the AP sliced meat. The MMNs of *E. coli* O157:H7 were 3.23, 4.18 and 5.36 log cfu/g on the MAP sliced meat stored at 6, 8 and 12°C respectively.

DISCUSSION

The growth characteristics of *E. coli* O157:H7 and AB on the packed sliced meat were influenced by the modified atmospheres and storage temperatures. The growth of *E. coli* O157:H7 occurred at 6-12°C with populations increase

Table 1. Means of the LPD (h), GR (log10 cfu/h) and MMN (log10 cfu/g) (standard deviation) of *E. coli* O157:H7 and aerobic bacteria (AB) in the packed sliced meat stored at 4-12°C.

Temperature (°C)	Microbial Count	AP			VP			MAP		
		LPD	GR	MMN	LPD	GR	MMN	LPD	GR	MMN
4	*E. coli*	75 (4)	0.0013 (0.0003)	3.38 (0.31)	81 (2)	-0.0015* (0.0001)	1.99 (0.42)	84 (4)	-0.0019 (0.0002)	1.76 (0.08)
	AB	72 (1)	0.0074 (0.0001)	7.89 (0.42)	76 (7)	0.0058 (0.0002)	7.07 (0.26)	78 (2)	0.0052 (0.0001)	6.77 (0.12)
6	*E. coli*	69 (3)	0.0021 (0.0001)	3.80 (0.26)	72 (4)	0.0015 (0.0002)	3.48 (0.40)	71 (4)	0.0010 (0.0003)	3.23 (0.34)
	AB	67 (1)	0.0080 (0.0002)	8.18 (0.31)	69 (3)	0.0066 (0.0003)	7.49 (0.26)	68 (6)	0.0059 (0.0004)	7.14 (0.29)
8	*E. coli*	50 (7)	0.0049 (0.0002)	5.18 (0.25)	55 (6)	0.0034 (0.0001)	4.47 (0.32)	54 (3)	0.0029 (0.0002)	4.18 (0.12)
	AB	46 (4)	0.0093 (0.0004)	8.84 (0.43)	53 (1)	0.0074 (0.0004)	7.91 (0.18)	51 (1)	0.0069 (0.0001)	7.64 (0.27)
12	*E. coli*	37 (5)	0.0088 (0.0116)	7.18 (0.51)	42 (5)	0.0063 (0.0002)	5.89 (0.12)	44 (1)	0.0052 (0.0004)	5.36 (0.28)
	AB	36 (4)	0.0167 (0.0009)	9.97 (0.23)	39 (2)	0.0081 (0.0011)	8.239 (0.36)	40 (2)	0.0074 (0.0012)	7.89 (0.34)

*Minus (-) indicates reduction.

from 2.74 to ≥3.80, ≥3.48 and ≥3.23 log cfu/g in the AP, VP and MAP sliced meat respectively. AB displayed similar growth patterns during storage periods. The packaging of the sliced meat with CO_2 and vacuum reduced the GRs and MMNs of *E. coli* and AB, and the influence diminished with the temperature increases. *E. coli* O157:H7 did not grow at 4°C in the VP and MAP sliced meat over 21 days of storage. The population showed an inactivetion rate of -0.0015 and -0.0019 log/h.

Temperature is probably the most important extrinsic factor affecting the growth and viability of microorganisms. Tamplin et al. (2005) reported the growth of *E. coli* O157:H7 at 6°C on the raw ground beef. During commercial storage, temperatures may fluctuate and opportunities exist for temperature abuse. At 4°C, an increase of *E. coli* O157:H7 was observed as the initial level of the bacterium rose by 0.48 log cfu/g after the 12 days holding period of lamp chops under aerobic condition (Barrera et al., 2007). Barkocy-Gallagher et al. (2002) observed minor increases for six *E. coli* O157:H7 strains on the ground beef held at 4°C for 14 days under aerobic condition, they significantly increased (between 0.9 and 1.5 log cfu/g) at 7°C. Many studies reported that *E. coli* O157:H7 on the inoculated raw meat significantly ($p<0.05$) increased at a temperature ranging from 10 to 15°C under aerobic conditions (Berry and Koohmaraie, 2001; Li and Logue, 2005; Tamplin et al., 2005; Barrera et al., 2007). In the present study, an increase of *E. coli* O157:H7 was observed as the initial level of the bacterium rose by 0.37 log cfu/g after 21 days storage at 4°C under aerobic condition.

Along with temperature, atmosphere gas composition is a major environmental factor that can influence microbial growth. The simplest form of modified atmosphere packaging is the vacuum packaging. In this study, the VP sliced meat storage at 4°C for 21 days resulted in a slight decrease (0.75 log cfu/g) of *E. coli* O157:H7 number while decrease was higher in MAP (0.78 log cfu/g). Two gases, CO_2 and N_2 are commonly used in MAP, the first being mainly responsible for the bacteriostatic or bactericidal effect on microorganisms. For meat and meat products, red color maintenance is important (Erkmen and Barazi, 2008; Erkmen and Bozoglu, 2008a). During storage of *E. coli* O157:H7 inoculated sliced meat at 4°C in 30% CO_2 atmosphere, the pathogen showed significant ($p<0.05$) reduction compared to similar conditions in air. To inhibit growth of spoilage and pathogenic bacteria and increase shelf life, retailers can use modification of atmosphere. *E. coli* O157:H7 shows growth at or over 6°C and is most relevant for packed meat and meat products (Rao and Sachindra, 2002; Barrera et al., 2007).

The present study shows that the prolonged shelf life at 4°C did not increase the risk of *E. coli* O157:H7 on the sliced meat stored with the vacuum or CO_2 packaging without the temperature abuse. This is probably due to the anaerobic conditions and high CO_2 that is inhibitory to *E. coli* O157:H7. The growth of *E. coli* O157:H7 in the VP

and MAP sliced meat from 6 to 12°C does however emphasize the importance of temperature control during storage. There is a wide range of temperature criteria for chilled foods at retail in European countries. The values range from -1 to 10°C, with most temperatures being between 4 and 8°C (European Commission, 1996). These aspects should also be considered together with the conclusions of the study, which states that modification of atmosphere has proven to enhance the product quality by inhibiting the spoilage and pathogenic bacteria. The data from this study add to the vast growth/survival data of *E. coli* O157:H7 on the sliced meat as effected by the storage temperature, modified atmosphere, and product handling and storage conditions. Temperature control is very important to ensure no growth of *E. coli* O157:H7 on the sliced meat with modification of storage atmosphere. Results from this study add to the understanding of the behavior of *E. coli* O157:H7 on the sliced meat and hence further enhance the safety of food prepared from this meat.

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